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Weissgerber, T. L., Gandley, R. E., Roberts, J. M., Patterson, C. C., Holmes, V. A., Young, I. S., McCance, D. R., & Diabetes and Pre-eclampsia Intervention Trial Study Group (2013). Haptoglobin Phenotype, Preeclampsia and Response to Supplementation with Vitamins C and E in Pregnant Women with Type 1 Diabetes. *BJOG: An International Journal of Obstetrics and Gynaecology*, *120*(10), 1192-1199. https://doi.org/10.1111/1471-0528.12288

Published in:
BJOG: An International Journal of Obstetrics and Gynaecology

Document Version:
Publisher's PDF, also known as Version of record

DOI: 10.1111/1471-0528.12288
www.bjog.org

Haptoglobin phenotype, pre-eclampsia, and response to supplementation with vitamins C and E in pregnant women with type-1 diabetes

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Accepted 30 March 2013. Published Online 30 May 2013.

Objective The phenotype of the antioxidant and pro-angiogenic protein haptoglobin (Hp) predicts cardiovascular disease risk and treatment response to antioxidant vitamins in individuals with diabetes. Our objective was to determine whether Hp phenotype influences pre-eclampsia risk, or the efficacy of vitamins C and E in preventing pre-eclampsia, in women with type-1 diabetes.

Design This is a secondary analysis of a randomised controlled trial in which women with diabetes received daily vitamins C and E, or placebo, from 8 to 22 weeks of gestation until delivery.

Setting Twenty-five antenatal metabolic clinics across the UK (in north-west England, Scotland, and Northern Ireland).

Population Pregnant women with type-1 diabetes.

Methods Hp phenotype was determined in white women who completed the study and had plasma samples available (n = 685).

Main outcome measure Pre-eclampsia.

Results Compared with Hp 2-1, Hp 1-1 (OR 0.59, 95% CI 0.30–1.16) and Hp 2-2 (OR 0.93, 95% CI 0.60–1.45) were not associated with significantly decreased pre-eclampsia risk after adjusting for treatment group and HbA1c at randomisation. Our study was not powered to detect an interaction between Hp phenotype and treatment response; however, our preliminary analysis suggests that vitamins C and E did not prevent pre-eclampsia in women of any Hp phenotype (Hp 1-1, OR 0.77, 95% CI 0.22–2.71; Hp 2-1, OR 0.81, 95% CI 0.46–1.43; Hp 2-2, 0.67, 95% CI 0.34–1.33), after adjusting for HbA1c at randomisation.

Conclusions The Hp phenotype did not significantly affect pre-eclampsia risk in women with type-1 diabetes.

Keywords Haptoglobin phenotype, pre-eclampsia, pregnancy, type-1 diabetes, vitamin C, vitamin E.

Please cite this paper as: Weissgerber T, Gandley R, Roberts J, Patterson C, Holmes V, Young I, McCance D. Haptoglobin phenotype, pre-eclampsia, and response to supplementation with vitamins C and E in pregnant women with type-1 diabetes. BJOG 2013;120:1192–1199.

Introduction

Pre-eclampsia affects 15–18% of women with type-1 diabetes,[1,2] leading to increased maternal and fetal morbidity and mortality.[3,4] Oxidative stress is associated with pre-eclampsia,[5,6] and the antioxidant vitamins C and E lowered pre-eclampsia incidence by 60% among high-risk women in a small randomised controlled trial (RCT).[7] Unfortunately, subsequent RCTs in high- and low-risk women,[8–13] and in women with type-1 diabetes,[14] were negative. Although these divergent results are likely to reflect low power in the small trial, differences could also be explained by the greater diversity of patients in multicentre trials masking a subset of responsive women.

Haptoglobin (Hp) is an antioxidant and pro-angiogenic protein,[15,16] with three generically determined phenotypes (1-1, 2-1, and 2-2).[17] Hp 1-1 is the strongest antioxidant.[15] Hp 2-2 is the most angiogenic.[16] Little is known about the function of Hp 2-1, which is structurally distinct from Hp 1-1 and Hp 2-2.[17] The Hp phenotype predicts cardiovascular risk,[18,19] and responsiveness to vitamin E,[20–22] or to vitamins C and E,[23] in individuals with diabetes.

We examined whether the Hp phenotype might affect pre-eclampsia risk, or identify women with type-1 diabetes who would respond to vitamin supplementation, for three reasons. First, angiogenic imbalance and oxidative stress contribute to pre-eclampsia,[5,6] and the pro-angiogenic and antioxidant properties of Hp are phenotype-dependant.[16,17] In our recent study, Hp 2-1 was associated with a two-fold greater pre-eclampsia risk among white women without diabetes.[24] Second, all three phenotypes are common in white women (17–48%); therefore, any effect would affect a large proportion of women.[25] Third, Hp phenotype influences cardiovascular risk, and responsiveness to vitamin E,[20–22] or to vitamins C and E,[23] in individuals with diabetes. The cardiovascular event risk is doubled in Hp 2-2 individuals with diabetes, compared with Hp 1-1 and Hp 2-1 individuals with diabetes,[20,23,26] and vitamin E eliminates this increased risk.[20,21,27] In contrast, vitamin C combined with vitamin E is either beneficial or harmful, depending on the Hp phenotype.[23] In postmenopausal women with coronary artery disease, vitamins C and E decreased coronary artery diameter in Hp 2-2 women with diabetes, but benefited Hp 1-1 women by increasing coronary artery diameter.[23]

We performed a secondary analysis of an RCT of antioxidants to prevent pre-eclampsia in women with type-1 diabetes to determine whether Hp phenotype is associated with pre-eclampsia risk, or antioxidant response, in these women.[14] We hypothesized that Hp 2-1 would be associated with an increased pre-eclampsia risk, in accordance with our previous data from women without diabetes. We also posited that the phenotype would affect treatment response, although the nature of the effect was difficult to predict using data from non-pregnant individuals.

Methods

Study population

This was a secondary analysis of a multicentre RCT (ISRCTN 27214045) in which 762 women with type-1 diabetes received 1000 mg of vitamin C and 400 iu of vitamin E, or placebo, daily from 8 to 22 weeks of gestation until delivery. The trial was conducted from 2003 to 2008 in 25 antenatal metabolic clinics across the UK (in north-west England, Scotland, and Northern Ireland). Full details have been reported previously.[14] The West Midlands multicentre research ethics committee approved the study (MREC 02/7/016). All subjects provided written, informed consent prior to participating. Hp phenotypes were determined at the University of Pittsburgh (Institutional Review Board exempt approval PRO10090150; retrospective analysis of samples already in existence).

The distribution of Hp phenotypes depends upon race.[25] In the Diabetes and Pre-eclampsia Intervention Trial (DAPIT), 96.5% of women were white ($n = 735$), 0.9% were black ($n = 7$), 1.4% were Asian ($n = 11$), and 1.2% were of other or unknown race ($n = 9$). The sample sizes were too small to draw conclusions about women who were not white; therefore, these women were excluded from the analysis. Fifty white women were excluded because plasma samples were not available. Hp phenotype was determined in the remaining 685 white women. Ten women who experienced fetal loss before 20 weeks of gestation were excluded from analyses examining the relationship between Hp phenotype, pre-eclampsia risk, and vitamin supplementation.

The primary outcome for the trial was pre-eclampsia, defined as gestational hypertension and proteinuria according to the International Society for the Study of Hypertension in Pregnancy guidelines at the time of the trial.[28] Gestational hypertension was defined as two diastolic blood pressure readings ≥90 mmHg, separated by at least 4 hours, or a single diastolic blood pressure reading ≥110 mmHg, between 20 weeks of gestation and 48 hours postpartum, excluding labour. Proteinuria was defined as a dipstick reading ≥1+ on at least two occasions, or a 24-hour urinary protein ≥300 mg. Among women with pre-existing proteinuria, pre-eclampsia was diagnosed if women had dipstick readings of ≥ 2+, 24-hour urinary protein ≥600 mg, or one or more other features of pre-eclampsia, such as those defining the HELLP syndrome (haemolysis, elevated liver enzymes, and low platelet count) or eclampsia. Secondary outcomes for this analysis were severe pre-eclampsia and early onset pre-eclampsia. Severe pre-eclampsia was defined as pre-eclampsia accompanied by one or more of the following: diastolic blood pressure ≥110 mmHg, the highest urine protein dipstick reading ≥3+, a urine output <500 ml in 24 hour, grand mal seizures, blurred vision, headache, pulmonary oedema, HELLP syndrome, or epigastric pain. Data for early onset pre-eclampsia are presented as pre-eclampsia with delivery before 34 or 37 weeks of gestation.

Haptoglobin phenotyping

Hp phenotype was determined as described previously.[24] Native polyacrilamide gel electrophoresis (PAGE) was performed on 5 μl of citrated plasma supplemented with 3 μl of 25 μmol/l human haemoglobin (Sigma-Aldrich, St Louis, MO, USA). Samples were run on 6% tris-glycine gels (Invitrogen, Carlsbad, CA, USA) for 2 hours at 120 V, then transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The peroxidase activity of the haemoglobin/Hp complex was used to visualise the Hp phenotype.

Samples that were haemolysed or had low Hp concentrations were phenotyped by SDS-PAGE.[24] A 2-μl portion of β-mercaptoethanol was added to 1–6 μl of serum. After heating for 7 minutes at 82°C, samples were run on 15%

tris-glycine gels at 120 V for 1.75 hours. Proteins were then transferred to PVDF. Membranes were incubated with blocking solution (tris-buffered saline containing 5% non-fat milk, 0.1% Tween 20), primary antibody (1:5000, polyclonal rabbit anti-human haptogloin; DakoCytomation, Carpinteria, CA, USA) and secondary antibody (1:25 000, goat anti-rabbit immunoglobulin G horseradish peroxidase; Millipore) at room temperature for 1 hour each. Antibodies were dissolved in blocking solution, and membranes were washed in tris-buffered saline containing 0.1% Tween 20 between incubations.

Membranes from native and SDS-PAGE were stained for peroxidase activity (SuperSignal West Pico Chemiluminescent Substrate; Fisher Scientific, Pittsburgh, PA, USA) and imaged (FlouroChem Q System; Cell Biosciences, Santa Clara, CA, USA). Hp phenotypes were identified by their characteristic banding patterns (Figure 1).

Statistical analysis

Quantitative variables were summarized using means and standard deviations unless distributions were heavily skewed, in which case medians and interquartile ranges were used. Comparisons of characteristics between Hp phenotype groups were performed using one-way analysis of variance, Kruskal–Wallis analysis of variance of ranks, or the chi-square test. Logistic regression analysis was used to compare the risks of pre-eclampsia in the phenotype groups. The 2-1 phenotype was selected as the reference category, as this was the phenotype with the largest sample size. Logistic regression was performed both before and after adjustment for two potential confounding factors, treatment group (vitamins or placebo) and HbA1c category at randomisation (≤6.0, 6.0–6.9, 7.0–7.9, ≥8.0%, or unknown). Logistic regression was also used to check if the effect of vitamin supplementation on pre-eclampsia risk differed between the three Hp phenotype groups by adding the interaction between Hp phenotype and treatment group to the model.

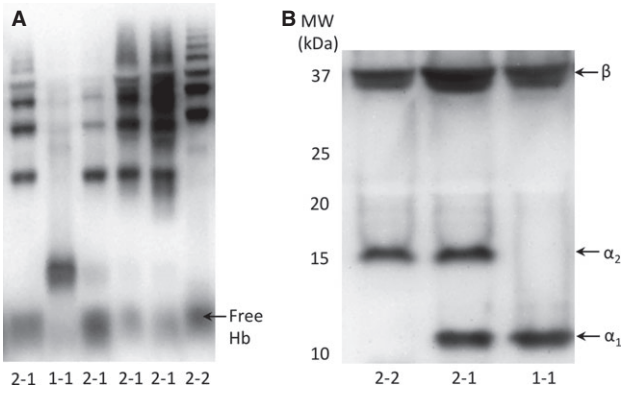

Figure 1. Hp phenotypes by Native and SDS PAGE. (A) Hp phenotyping of Hb-supplemented plasma by Native PAGE on a 6% gel using Hb peroxidase detection. Hp 1-1 has a single fast-migrating band (lane 2). Hp 2-2 has a series of slowly migrating bands (lane 6). Hp 2-1 (lanes 1 and 3–5) has one band between the Hb/Hp 1-1 and Hb/Hp 2-2 bands, and several slowly migrating bands in the same region as the Hb/Hp 2-2 bands. (B) Hp phenotyping of plasma by SDS-PAGE on a 12% gel with western blotting. All phenotypes have the Hp β band. The α_1 band indicates that the Hp 1 allele is present. The α_2 band indicates that the Hp 2 allele is present. Hp 2-2 has α_2 and β bands (lane 1), Hp 2-1 has α_1, α_2 and β bands (lane 2), and Hp 1-1 has α_1 and β bands (lane 3).

Results

Subject characteristics

The prevalence of the Hp 1-1 (14.7%), Hp 2-1 (48.7%), and Hp 2-2 (36.6%) phenotypes were similar to previously reported values for white men and women.[25] Women with the Hp 2-1 phenotype were randomised slightly earlier than women with the Hp 1-1 or Hp 2-2 phenotypes, and this small difference was the only statistically significant difference ($P < 0.05$) found in 19 comparisons (Table 1). Hp phenotype groups did not differ with respect to maternal demographic characteristics (age, parity, and education), physical characteristics (body mass index, blood pressure, and albumin-creatinine ratio at randomisation), health behaviours (smoking, and consumption of multivitamins or aspirin prior to randomisation), diabetes history (duration, HbA1c, and insulin dose at randomisation), or hypertension (hypertension or antihypertensive treatment before pregnancy, previous pre-eclampsia).

Hp phenotype and pre-eclampsia risk

There was no significant difference in the risk of pre-eclampsia, severe pre-eclampsia, or early onset pre-eclampsia between the three phenotype groups (Table 2). Compared with Hp 2-1, Hp 1-1 and Hp 2-2 were not associated with a significantly decreased risk of pre-eclampsia, severe pre-eclampsia, or early onset pre-eclampsia (Table 3). Adjustment for treatment group (vitamins C and E versus placebo) and HbA1c category at randomisation had a minimal effect on the odds ratios.

Hp phenotype and treatment response

There was no significant interaction between the effect of Hp phenotype and the effect of vitamin supplementation on pre-eclampsia risk ($P = 0.87$). Odds ratios for the development of pre-eclampsia with vitamin supplementation were 0.67 (95% CI 0.15–2.67) in Hp 1-1, 0.82 (95% CI 0.45–1.51) in Hp 2-1, and 0.66 (95% CI 0.32–1.36) in Hp 2-2 (Table 4). Supplementation with vitamins C and E did not significantly reduce pre-eclampsia risk in white women with type-1 diabetes of any Hp phenotype.

Table 1. Maternal demographic characteristics

Variable	Hp 1-1 (*n* = 99)	Hp 2-1 (*n* = 335)	Hp 2-2 (*n* = 251)	*P*
Age (years)*	29.7 ± 5.7	29.6 ± 5.5	29.8 ± 5.6	0.96
Gestational age at randomisation (weeks)*	14.2 ± 3.3	13.7 ± 3.3	14.4 ± 3.6	0.05
Body mass index at randomisation (kg/m^2)*	28.0 ± 5.2	27.8 ± 5.5	27.1 ± 4.2	0.15
Current smoker**	21 (21%)	75 (22%)	40 (16%)	0.14
Primiparous**	44 (44%)	170 (51%)	118 (47%)	0.46
12 years or fewer of full-time education**	36 (36%)	132 (39%)	96 (38%)	0.85
Taking multivitamin at randomisation**	12 (12%)	33 (10%)	26 (10%)	0.81
Taking aspirin at randomisation**	5 (5%)	23 (7%)	18 (7%)	0.77
Antihypertensive treatment before pregnancy**	7 (7%)	28 (8%)	24 (10%)	0.74
Hypertension before pregnancy**	18 (18%)	49 (15%)	37 (15%)	0.68
Blood pressure at entry (8–22 weeks; mmHg)				
Systolic*	120.1 ± 12.3	118.0 ± 11.6	119.9 ± 12.1	0.11
Diastolic*	76.1 ± 8.8	74.1 ± 8.1	75.1 ± 9.0	0.09
Pre-eclampsia in previous pregnancy**	14 (14%)	40 (12%)	22 (9%)	0.28
Diabetes				
Duration (years)*	14.6 ± 7.9	14.1 ± 8.0	15.1 ± 8.5	0.38
HbA1c at randomisation*	7.3 ± 1.1	7.2 ± 1.0	7.1 ± 0.9	0.47
Insulin at randomisation (units/day)***	60 (43–74)	58 (42–74)	54 (44–68)	0.24
Renal status before index pregnancy***				
Normal	87 (88%)	307 (92%)	230 (92%)	0.56
Microalbuminuria	7 (7%)	14 (4%)	11 (4%)	
Macroalbuminuria	1 (1%)	3 (1%)	0 (0%)	
Urinary protein >3 g/24 hours	0 (0%)	2 (1%)	2 (1%)	
Not known	4 (4%)	9 (3%)	8 (3%)	
Albumin:creatinine at randomisation (mg/mmol)***	0.81 (0.46–1.98)	0.69 (0.36–1.47)	0.70 (0.41–1.53)	0.20

Values are means ± SDs, median (interquartile range) or *n* (%).
*One-way ANOVA.
**Chi-square test.
***Kruskal–Wallis ANOVA of ranks.

Table 2. Pre-eclampsia incidence according to Hp phenotype

Outcome	Hp 1-1 (*n* = 99)	Hp 2-1 (*n* = 329)	Hp 2-2 (*n* = 247)	*P*
Pre-eclampsia	12 (12.1%)	59 (17.9%)	42 (17.0%)	0.39
Severe pre-eclampsia	10 (10.1%)	53 (16.1%)	33 (13.4%)	0.29
Early onset pre-eclampsia				
<34 weeks	2 (2.0%)	12 (3.6%)	6 (2.4%)	0.58
<37 weeks	10 (10.1%)	41 (12.5%)	24 (9.7%)	0.55

Values are *n* (%). Groups were compared using the chi-square test.

Discussion

This secondary analysis of an RCT of daily supplementation with vitamins C and E to prevent pre-eclampsia reveals two important findings.[14] First, Hp phenotype was not associated with pre-eclampsia risk in white women with type-1 diabetes. Second, although our study was not powered to detect an interaction between Hp phenotype and treatment, we found no evidence that vitamins C and E significantly affect pre-eclampsia risk in women of any Hp phenotype.

Hp phenotype and pre-eclampsia risk

When we began this investigation, two small, underpowered case–control studies in women without diabetes had reported that pre-eclampsia risk was either increased or did not differ in Hp 1-1 women.[29,30] Cohort studies, adequately powered studies, and studies in women with diabetes were needed. A subsequent Israeli study in women without diabetes reported a lower pre-eclampsia risk among Hp 1-1 women, compared with Hp 2-1 and Hp 2-2 women (5.8 versus 12.5%).[31] Our larger case–control study indicated that Hp 1-1 was protective in comparison with Hp 2-1, but not with Hp 2-2, in white women without diabetes.[24] Racial differences between populations may have contributed to these divergent results in women without diabetes; however, the small sample sizes of early studies suggest that spurious findings may also have been a factor.

Table 3. Odds ratios for pre-eclampsia

Outcome	Phenotype	*n* (% within group)		OR (95% CI)*	Adjusted OR (95% CI)*,**
		Pre-eclampsia	Controls		
Pre-eclampsia	1-1	12 (10.6%)	87 (15.5%)	0.63 (0.32–1.23)	0.59 (0.30–1.16)
	2-1	59 (52.2%)	270 (48.0%)	1	1
	2-2	42 (37.2%)	205 (36.5%)	0.94 (0.61–1.45)	0.93 (0.60–1.45)
Severe pre-eclampsia	1-1	10 (10.4%)	87 (15.5%)	0.59 (0.29–1.20)	0.55 (0.27–1.13)
	2-1	53 (55.2%)	270 (48.0%)	1	1
	2-2	33 (34.4%)	205 (36.5%)	0.82 (0.51–1.31)	0.81 (0.50–1.31)
Early onset pre-eclampsia (<34 weeks)	1-1	2 (10.0%)	87 (15.5%)	0.52 (0.11–2.36)	0.47 (0.10–2.15)
	2-1	12 (60.0%)	270 (48.0%)	1	1
	2-2	6 (30.0%)	205 (36.5%)	0.66 (0.24–1.78)	0.68 (0.25–1.85)
Early onset pre-eclampsia (<37 weeks)	1-1	10 (13.3%)	87 (15.5%)	0.76 (0.36–1.57)	0.72 (0.35–1.51)
	2-1	41 (54.7%)	270 (48.0%)	1	1
	2-2	24 (32.0%)	205 (36.5%)	0.77 (0.45–1.32)	0.77 (0.45–1.32)

Statistical analysis was performed by logistic regression.
*Hp 2-1 was used as the reference group for all comparisons.
**Adjusted for treatment group and HbA1c at randomisation by category (≤6.0%, 6.0–6.9%, 7.0–7.9%, ≥8.0%, and unknown).

Table 4. Incidence of pre-eclampsia among women in the placebo and treatment groups

Phenotype	Treatment	Placebo	OR (95% CI)	*P*	Adjusted OR (95% CI)*	*P*
1-1	5/50 (10.0%)	7/49 (14.3%)	0.67 (0.20–2.26)	0.52	0.77 (0.22–2.71)	0.69
2-1	26/158 (16.5%)	33/171 (19.3%)	0.82 (0.47–1.45)	0.50	0.81 (0.46–1.43)	0.46
2-2	18/127 (14.2%)	24/120 (20.0%)	0.66 (0.34–1.29)	0.23	0.67 (0.34–1.33)	0.25

Statistical analysis was performed by logistic regression.
*Adjusted for HbA1c at randomisation by category (≤6.0%, 6.0–6.9%, 7.0–7.9%, ≥8.0%, and unknown).

The present study extends the existing literature to include women with type-1 diabetes. This is particularly important, as the impact of Hp phenotype on cardiovascular disease risk,[18,19] and on responsiveness to antioxidant vitamins,[20–23] in non-pregnant populations is primarily confined to individuals with diabetes. In the present study, Hp phenotype was not associated with the risk of pre-eclampsia, severe pre-eclampsia, or early onset pre-eclampsia in white women with type-1 diabetes. Rates of pre-eclampsia tended to be lower in Hp 1-1 women; however, this effect was clearly not significant. Although the sample size for this secondary analysis was limited by the low number of participants in the RCT, the number of subjects was sufficient to detect a 14% difference in pre-eclampsia risk between Hp 1-1 and Hp 2-1 (12 versus 26%; 80% power; $\alpha = 0.05$). The study was not powered to detect smaller differences. Despite this limitation, this is the only study we are aware of that has examined women with type-1 diabetes. The cohort design is also a significant strength, as all studies in women without diabetes have used a case–control design.

Hp phenotype and vitamins C and E

Emerging evidence suggests that there is a strong relationship between pre-eclampsia and future cardiovascular disease. Pre-eclampsia and cardiovascular disease share many risk factors (i.e. obesity, diabetes, being black, and hyperlipidaemia) and underlying pathophysiological processes (endothelial dysfunction, angiogenic imbalance, inflammation, and oxidative stress).[32,33] Pre-eclampsia is now recognised as a risk factor for cardiovascular disease by the American Heart Association.[33] Hp phenotype affects both cardiovascular disease risk and treatment response to antioxidant vitamins in individuals with diabetes.[20,21,23,26] Therefore, we examined the potential for Hp to modify the efficacy of vitamins C and E in preventing pre-eclampsia in women with type-1 diabetes.

Our study did not have sufficient power to detect an interaction between Hp phenotype and treatment response; however, our preliminary analysis provided no evidence that supplementation with vitamins C and E prevented pre-eclampsia among white women with type-1 diabetes of any Hp phenotype. It is extremely unlikely that larger

studies will ever be conducted. Several factors could contribute to the difference between our study and previous studies of cardiovascular disease risk in individuals with diabetes.[20,21,23,26] First, although pre-eclampsia and cardiovascular disease share many risk factors and pathophysiological processes, there are important differences between these two conditions. Pre-eclampsia is a pregnancy-specific syndrome, in which one or more factors released by the placenta are believed to contribute to maternal vascular dysfunction.[32] Factors that are only produced by the placenta, or are released from the placenta in larger quantities than from other tissues, are unlikely to play a major role in cardiovascular disease.

Second, studies of cardiovascular disease suggest that the effects of vitamin E alone differ from the effects of combined vitamin C and E supplementation.[20,21,27] Among postmenopausal women with coronary artery disease, vitamins C and E may be beneficial or harmful depending on Hp phenotype.[23] Supplementation increased coronary artery diameter in Hp 1-1 women, and this beneficial effect was stronger in Hp 1-1 women with diabetes.[23] In Hp 2-2 women with diabetes, however, the weak antioxidant capacity of Hp 2-2 may have interacted with the pro-oxidant effects of vitamin C to accelerate coronary artery narrowing.[23,24] Both type-1 diabetes and pregnancy increase oxidative stress,[32,35] suggesting that a similar mechanism could potentially be active in our study population; however, we did not find evidence to support this hypothesis.

Third, previous studies examining the interaction between Hp phenotype and responsiveness to antioxidant vitamins have focused on type-2 diabetes.[21,26] Women in the present study had type-1 diabetes. Hp 2-2 increases cardiovascular disease risk in both type-1 and type-2 diabetes.[19,20] The purported mechanisms by which vitamin E reduces risk (by modifying the adverse effects of glycosylated haemoglobin on high-density lipoprotein dysfunction[34]) is likely to be similar in both conditions; however, the relationship between Hp phenotype, risk of cardiovascular disease and vitamin supplementation in type-1 diabetes has not yet been investigated.

Conclusion

In contrast to the results of previous studies in women without diabetes,[24,31] Hp phenotype did not significantly affect pre-eclampsia risk in white women with type-1 diabetes. Researchers have suggested that Hp phenotype may identify a subgroup of women who would benefit from antioxidant supplementation to prevent pre-eclampsia.[24,36] Although our study had limited power to detect an interaction between Hp phenotype and treatment response, our preliminary analysis did not suggest that supplementation with vitamins C and E would modify pre-eclampsia risk in women with type-1 diabetes of any Hp phenotype. It remains possible that other types or combinations of antioxidants may benefit women of specific Hp phenotypes; however, the absence of a relationship between Hp phenotype and pre-eclampsia risk makes this possibility unlikely.

Disclosure of interests

None of the authors have any conflicts of interest.

Contribution to authorship

TLW conceived and designed the research, performed the Hp phenotyping, and drafted the article. TLW, REG, VAH, DRM, ISY, and JMR acquired the data. CCP performed the statistical analyses. All authors analysed and interpreted the data and edited and revised the article.

Details of ethics approval

The West Midlands multicentre research ethics committee approved the study (MREC 02/7/016). All subjects provided written, informed consent prior to participating. Hp phenotypes were determined at the University of Pittsburgh (Institutional Review Board exempt approval PRO10090150, 26 January 2011; retrospective analysis of samples in existence).

Funding

DAPIT was funded by grant 067028/Z/02/Z and grant 083145/Z/07/Z from The Wellcome Trust (registered charity number 210183). This work was supported by NIH P01 HD030367. T.L.W. was supported by a Canadian Institute of Health Research Fellowship, an Amy Roberts Health Promotion Research Award, and the Office of Women's Health Research (Building Interdisciplinary Careers in Women's Health award K12HD065987).

Acknowledgements

The authors thank Cyril McMaster from the Centre for Public Health, Queen's University Belfast, UK, for his assistance with sample sorting and shipping. The authors are grateful to the patients who took part in DAPIT, the DAPIT research midwives who collected the data, and the collaborators at each centre. In addition to the authors, members of the DAPIT Study Group are: Dr Michael J.A. Maresh, Department of Obstetrics and Gynaecology, St Mary's Hospital for Women and Children, Manchester, UK; Prof Donald Pearson, Department of Diabetes, Aberdeen Royal Infirmary, Aberdeen, UK; and Dr James Walker, Department of Diabetes, St John's Hospital at Howden, West Lothian, UK. ■

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Commentary on 'Haptoglobin phenotype, pre-eclampsia, and response to supplementation with vitamins C and E in pregnant women with type–1 diabetes'

Weissgerber and colleagues have reported a secondary biologic sample analysis from a large, multicentre clinical trial of antioxidants for the prevention of pre-eclampsia in pregnant women with type–1 diabetes. Unfortunately, antioxidants have now joined the list of failed pre-eclampsia prevention therapies, with numerous trials of high- and low-risk women demonstrating no preventive benefit. That said, however, there still exists the possibility that antioxidants might prove useful in appropriately identified subsets of the obstetric population. One such subset could be women with type–1 diabetes. Although almost all of the high- and low-risk antioxidant trials specifically excluded these women, the parent trial from which these data and samples were derived (McCance DR *et al. Lancet* 2010;376:259–66) specifically studied antioxidant pre-eclampsia prevention in pregnant type–1 diabetic women.

Haptoglobin (Hp) is an antioxidant protein that has two allelic forms and three genotypes. These three genotypes have varying antioxidant potentials that, in non-pregnant diabetics, predict cardiovascular risk and antioxidant responsiveness. Unfortunately, the current study demonstrates that maternal Hp genotype does not identify any subsets of the pregnant diabetic population that might benefit from antioxidant prophylaxis. The authors appropriately note that their statistical power is limited, but also comment that larger antioxidant trials in pregnant diabetic populations are very unlikely to be performed in the future.

In my opinion, this article has several important messages:

1. It is important to include diabetics in clinical trials. Although the proportion of pregnancies complicated by type–1 diabetes is well below 1%, these pregnancies consume disproportionate resources and represent substantial cost centres. We owe it not only to these women and their families, but to our respective health care systems, to specifically include them in clinical trials in order to optimise their outcomes.
2. Genomic medicine is poised to make substantial inroads to obstetric practice. Essentially all of the 'great obstetric syndromes' (certainly including pre-eclampsia) are final common pathways for many different etiologies, some of which are recognized, and many of which are still unknown. As these pathophysiologic mechanisms are elucidated, personalised preventions and treatments, like the hypothesis of this study, will rapidly proliferate. Likewise, over the next few decades pharmacogenomics will surely improve therapeutic outcomes and reduce adverse drug reactions.
3. Clinical trial designers should always consider including in the consent process the possibility of biologic sample banking (with the appropriate specification of uses) and of subsequent participant re-contact. This study was made possible by the foresight of the investigators of the parent study, who provided the opportunity for the testing of multiple secondary hypotheses, many of which would not have been imaginable at the time the parent study was designed. Access to these resources must also be as open as possible, consistent with viable scientific hypotheses.

The design of the parent study (focusing on diabetics), the accessibility of the data and biologic samples from the parent study for viable secondary analyses, and the application of genomic testing to obstetric care all represent important clinical research tenets that will contribute to improved outcomes and sustainable costs going forwards. ■

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